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Applicant: Lorin R. DeBonte et al.

Serial No.: 08/572,027

Filed : December 14, 1995

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Attorney's Docket No.: 07148-032001 / CGL99/0007US5A

## Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

# **Listing of Claims**:

## 1-9. (Canceled)

10. (Previously Presented) The nucleic acid fragment of claim 66, wherein said at least one mutation comprises a codon encoding His in place of the codon encoding Leu.

### 11.-26. (Canceled)

27. (Currently amended) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a full-length Brassicaceae delta-15 fatty acid desaturase gene coding sequence having at least one mutation in a region of said desaturase gene coding sequence encoding a His-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase gene coding sequence non-functional and wherein said sequence includes said at least one mutation.

#### 28. (Canceled)

- 29. (Previously Presented) The nucleic acid fragment of claim 27, wherein said sequence encodes a microsomal gene product.
  - 30. (Canceled)
- 31. (Previously Presented) The nucleic acid fragment of claim 27, wherein said at least one mutation introduces a non-conservative amino acid substitution in said region.

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- 32. (Previously Presented) The nucleic acid fragment of claim 31, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).
- 33. (Previously Presented) The nucleic acid fragment of claim 32, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.
- 34. (Currently amended) The nucleic acid fragment of claim 27, wherein said mutant desaturase gene coding sequence is from a *Brassica napus* plant.
- 35. (Previously Presented) A Brassicaceae plant containing a full-length coding sequence of a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.
  - 36. (Canceled)
- 37. (Previously Presented) The plant of claim 35, wherein said mutation confers a decreased level of  $\alpha$ -linolenic acid in seeds of said plant.
- 38. (Original) The plant of claim 35, wherein said mutant desaturase gene encodes a microsomal gene product.
- 39. (Original) The plant of claim 35, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.
- 40. (Previously Presented) The plant of claim 39, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

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- 41. (Previously Presented) The plant of claim 40, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.
- 42. (Original) The plant of claim 35, wherein said mutant desaturase gene is from a Brassica napus plant.
  - 43. (Original) The plant of claim 35, wherein said plant is a Brassica napus plant.
  - 44. (Previously Presented) A Brassicaceae plant containing:
  - a) a full-length coding sequence from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one delta-12 gene mutation in a region encoding a His-Xaa-Xaa-His amino acid motif; and
  - a full-length coding sequence from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-His amino acid motif;

wherein said delta-12 gene mutation and said delta-15 gene mutation render the products of said delta-12 desaturase gene and said delta-15 desaturase gene, respectively, non-functional.

- 45. (Previously Presented) The plant of claim 44, wherein said mutant genes confer a decreased level of  $\alpha$ -linolenic acid in seeds of said plant compared to  $\alpha$ -linolenic acid levels in corresponding seeds lacking said mutant genes.
- 46. (Previously Presented) A Brassicaceae or Helianthus plant containing a full-length coding sequence of a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

47.-54. (Canceled)

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55. (Previously Presented) A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
- b) obtaining one or more plants from said cells;
- c) identifying at least one of said plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation in said delta-12 gene renders the product of said delta-12 desaturase gene non-functional; and
- d) producing said plant line from said at least one plant, said plant line having said at least one mutation in said delta-12 gene.
- 56. (Original) The method of claim 55, wherein said plant line yields an oil having a stabilized linoleic acid content from about 2.0 % to about 12.0 %.
  - 57. (Previously Presented) The method of claim 55, further comprising the steps of:
    - e) inducing mutagenesis in cells of said plant line;
    - f) obtaining one or more plants from said cells of said plant line;
    - g) identifying at least one of said plants from step f) that contains a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation in said delta-15 gene is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 desaturase gene non-functional; and
    - h) producing a second plant line from said at least one plant identified in step g), said second plant line having said at least one mutation in said delta-12 gene and said at least one mutation in said delta-15 gene.

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- 58. (Original) The method of claim 55, wherein said starting variety is a Brassica napus variety.
- 59. (Original) The method of claim 58, wherein said mutation is in a first form of delta-12 fatty acid desaturase.
- 60. (Original) The method of claim 59, further comprising the step of crossing a plant of said plant line to a plant having a mutation in a second form of delta-12 fatty acid desaturase.
- (Original) The method of claim 60, wherein said second mutation is in a region 61. other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.
  - б2. (Previously Presented) The method of claim 59, further comprising the steps of:
  - e) inducing mutagenesis in cells of said plant line;
  - f) obtaining one or more plants from said cells of said plant line;
  - g) identifying at least one of said plants from step f) that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif: and
  - h) producing a second plant line from said at least one plant identified in step g), said second plant line having said first and second delta-12 gene mutations.
- 63. (Original) The method of claim 55, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.
- 64. (Previously Presented) A method for producing a Brassicaceae plant line. comprising the steps of:

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 inducing mutagenesis in cells of a starting variety of a Brassicaceae or Helianthus species;

- b) obtaining one or more plants from said cells;
- c) identifying at least one of said plants that contains a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said delta-15 desaturase gene non-functional; and
- d) producing said plant line from said at least one plant, said plant line having said mutation in said delta-15 gene.
- 65. (Original) The method of claim 64, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.
- 66. (Currently amended) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a full-length Brassicaceae or Helianthus delta-12 fatty acid desaturase gene coding sequence having at least one mutation in a region of said desaturase gene coding sequence encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif, wherein said at least one mutation renders the product of said desaturase gene coding sequence non-functional and wherein said sequence includes said at least one mutation.
- 67. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* plant, comprising:
  - a) providing a *Brassicaceae* plant having a decreased  $\alpha$ -linolenic acid content as compared with a corresponding control *Brassicaceae* plant; and
  - b) identifying at least one mutation in a delta-15 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 fatty acid desaturase gene non-functional.

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- 68. (Previously Presented) The method of claim 67, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.
- 69. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* or *Helianthus* plant, comprising:
  - a) providing a *Brassicaceae* or *Helianthus* plant having an increased oleic acid content as compared with a corresponding control *Brassicaceae* or *Helianthus* plant; and
  - b) identifying at least one mutation in a delta-12 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-12 fatty acid desaturase gene non-functional.
- 70. (Previously Presented) The method of claim 69, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.